

USSN - 09/896,095

rest of XI - T S S stop--

Please replace the paragraph on page 216, lines 29-31, with the following rewritten paragraph:

--(L) K K S - rest of VIII
ACT.TCC.AG.CTG.AAA.AAG.TCT. (SEQ ID NOs: 98, DNA,
and 99)

rest of XI -T S S stop--

The paragraph on page 26, lines 11-18, has been amended as follows:

Figure 15 shows the main chain of scorpion toxin (Brookhaven Protein Data Bank entry 1SN3) residues 20 through 42 (SEQ ID NO:274). CYS₂₅ and CYS₄₁ are shown forming a disulfide. In the native protein these groups form disulfides to other cysteines, but no main-chain motion is required to bring the gamma sulphurs into acceptable geometry. Residues, other than GLY, are labeled at the β carbon with the one-letter code.

The paragraph on page 110, lines 7-9, has been amended as follows:

5 6 6 7 7
5 0 5 0 3
MVVVIVGATIGIKLFKKFTSKAS (SEQ ID NO:122)

The paragraph on page 151, lines 33-35, has been amended as follows:

10 11 11 12 12 13
5 0 5 0 5 0
EYIGYAWAMVVVIVGATIGIKLFKKFTSKAS (SEQ ID NO:273)

The paragraph on page 154, lines 13-14 14, has been amended as follows:

84 85 86 87 88 89 90 91
GGT|GAT|GAT|CCG|GCC|AAA|GCG|GCC|gcg|cc 3' olig#12 (SEQ
ID NO:123)

The paragraph on page 154, lines 23-24, has been amended as follows:

88 89 90 91
GCC|AAA|GCG|GCC|gcg|cc 3' olig#12a (SEQ
ID NO:124)

The paragraph on page 154, lines 32-33, has been amended as follows:

88 89 90 91
GCC|AAA|GCG|GCC|gcg|cc 3' olig#12b (SEQ
ID NO:125)

The paragraph on page 154, lines 35-36, has been amended as follows:

residue 91 90 89 88 87 86
5' gg|cgc|GGC|CGC|TTT|GGC|CGG|ATC 3' olig#13 (SEQ ID NO:126)

The paragraph on page 203, lines 12-13, has been amended as follows:

Original putative RBS (5'-to-3')

GAGCTCaggaggCTTACTATGAAGAAATCTCTGGTTCTTAAGGCTAGC (SEQ ID NO:130)
SacI Nhe I

The paragraph on page 203, lines 16-17, has been amended as follows:

New RBS (5'-to-3')

GAGCTCTggaggAAATAAAATGAAGAAATCTCTGGTTCTTAAGGCTAGC
SacI Nhe I
(SEQ ID NO:131)

The paragraph on page 204, lines 2-6, has been amended as follows:

MB16

5' - GATCC tcttagtgccggc TTTACA ctttatgcttc(cg-gctcg..-3'
3' - G agatctcagccg aaatgt gaaatacgaag gc(cgagc..-5'
BamHI -35 HpaII

(SEQ ID NOs:132 (top), 133 (bottom))

The paragraph on page 204, line 9, has been amended as follows:

MB22 insert

5'- GATCC actccccatccccctg TTGACA attaatcat -3'
3'- G tgaggggtagggggac AACTGT taatttagtagc-5'
| -35 | |
BamHI (HpaII)

(SEQ ID NOs:134 (top), 135 (bottom))

The paragraph on page 207, lines 28-30, has been amended as follows:

.AAA.GCC.CGT.CCG.GAT.-3' (SEQ ID NO:136)
lys ala arg pro asp..... (SEQ ID NO:137)
|AccIII|

The paragraph on page 208, lines 33-34, has been amended as follows:

GCT.CAT.CCG.-3' (SEQ ID NO:138)
ala his pro.... (SEQ ID NO:139)

The paragraph on page 222, line 32, has been amended as follows:

5'-gtttcagcggCgCCagaatagaaag-3', (SEQ ID NO:140)

The paragraph on page 224, lines 1-3, has been amended as follows:

5'-TATTCTGGCGCCGT -3' (SEQ ID NO:141)
3'-ATAAGACCGCGGGCAGGCC-5' (SEQ ID NO:142)
|NarII| |AccIII|

The paragraph on page 317, line 24-page 318, line 9, has been amended as follows:

One mini-protein motif of potential use comprises a disulfide loop enclosing a helix, a turn, and a return strand.

Such a structure could be designed or it could be obtained from a protein of known 3D structure. Scorpion neurotoxin, variant 3, (ALMA83a, ALMA83b) (hereafter ScorpTx) contains a structure diagrammed in Figure 15 (SEQ ID NO:274) that comprises a helix (residues N22 through N33), a turn (residues 33 through 35), and a return strand (residues 36 through 41). ScorpTx contains disulfides that join residues 12-65, 16-41, 25-46, and 29-48. CYS₂₅ and CYS₄₁ are quite close and could be joined by a disulfide without deranging the main chain. Figure 15 shows CYS₂₅ joined to CYS₄₁. In addition, CYS₂₉ has been changed to GLN. It is expected that a disulfide will form between 25 and 41 and that the helix shown will form; we know that the amino-acid sequence shown (SEQ ID NO:274) is highly compatible with this structure. The presence of GLY₃₅, GLY₃₆, and GLY₄₀ give the turn and extended strand sufficient flexibility to accommodate any changes needed around CYS₄₁ to form the disulfide.

The paragraph on page 334, lines 49-52, has been amended as follows:

k	a	s	(SEQ ID NO:122)
129	130	131	132	133	134		
A.A.r G.C.n T.C.n T.A.r T.A.r T.A.r					(SEQ ID NO:143)		
	A.G.y T.G.A T.G.A T.G.A						

At the bottom of page 349, as a new paragraph, please add the following:

Homologues 1-19 are SEQ ID NOS:144-162, respectively.

At the bottom of page 351, as a new paragraph, please add the following:

Homologues 20-35 are SEQ ID NOS:163-178, respectively.

At the bottom of page 353, as a new paragraph, please add the following:

Homologues 36-40 are SEQ ID NOS:179-183, respectively.

At page 362, after the line beginning "ALA58", please add the following as a new paragraph:

(SEQ ID NO:144)

The paragraph on page 366, lines 42-48, has been amended as follows:

<u>+Sfi</u> I	GGCCNNNNNGGCC	P	8	&	5	<N,P,T	(SEQ ID NO:184) +
<u>Sma</u> I	CCCGGG	P	3	&	3	<B,M,I,N,P,T	
<u>Spe</u> I	ACTAGT	P	1	&	5	<M,N,T	
<u>Sph</u> I	GCATGC	P	5	&	1	<B,M,I,N,P,T	
<u>Stu</u> I	AGGCCT	P	3	&	3	<M,N,I(<u>Aat</u> I),P,T	
<u>%Sty</u> I	CCWWGG	P	1	&	5	<N,P,T	
<u>Xba</u> I	GTATAC	P	3	&	3	<N (soon)	

On page 368, at the end, as a new paragraph, please add the following:

(SEQ ID NO:185)

On page 369, before the last line, please add the following as a new paragraph:

(SEQ ID NO:185)

The paragraphs on page 374, lines 14-21 (ignoring blank lines) have been amended as follows:

| s | . | . | . | (SEQ ID NO:187)
| 131|132|133|134|
| TCT|TAA|TAG|TGA|GGT|TAC|CAG|TCT| 502

| BstE III |

| AAG|CCC|GCC|TAA|TGA|GCG|GGC|TTT|TTT|TTT| 532
| Trp terminator |

| CCT|GAG|G -3' (SEQ ID NO:186) 539
| Sau I |

The paragraphs on page 375, lines 14-21 (ignoring blank lines), have been amended as follows:

| s | . | . | . | (residues 128-131 of SEQ ID NO:187)
| 131 | 132 | 133 | 134 |

| TCT | TAA | TAG | TGA | GGT | TAC | CAG | TCT |
| BstE III |

| AAG | CCC | GCC | TAA | TGA | GCG | GGC | TTT | TTT | TTT |
| Trp terminator |

| CCT | GAG | GCA | ggt | gag | cg - 3' (SEQ ID NO:188)
| Sau I | spacer |

The paragraph on page 376, lines 15-16 (ignoring blank lines), has been amended as follows:

| CCT | GAG | GCA | GGT | GAG | CG (SEQ ID NO:189)
| gga ctc cgt cca ctc gc - 5' (SEQ ID NO:190)

The paragraph on page 377, last four lines, has been amended as follows:

| t | s | k | (SEQ ID NO:192)
| 127 | 128 | 129 |
| ACT | TCG | AAa | gcg | gct | gcg | - 3' (SEQ ID NO:191)
| Asu II | spacer |

The paragraph on page 378, lines 14-15 (ignoring blank lines), has been amended as follows:

| ACT | TCG | AAA | GCG | GCT | GCG | (SEQ ID NO:193)
| tga agc ttt cgc cga cgc - 5' (SEQ ID NO:194)

The paragraph on page 379, last four lines, has been amended as follows:

| t | s | k | (SEQ ID NO:196)
| 127 | 128 | 129 |
| ttt | acT | TCG | AAa | gcg | tcg | ccg | - 3' (SEQ ID NO:195)
| Asu II |

The paragraph on page 380, lines 13-14, has been amended as follows:

| TTT|ACT|TCG|AAA|GCG|TCG|CCG| (SEQ ID NO:197)
aaa tga agc ttt cgc agc ggc -5' (SEQ ID NO:198)

The paragraph on page 381, last 4 lines, has been amended as follows:

| k | l | f | k | k | f | t | s | k | (SEQ ID NO:200)
|121|122|123|124|125|126|127|128|129|
|AAA|CTG|TTT|AAG|AAA|TTT|ACT|TCG|AAa|gcg|tcg|ggc| - 3' (SEQ
ID NO:199)

Asu III spacer

The paragraph on page 382, lines 12-13 (not counting blank lines), has been amended as follows:

| AAA|CTG|TTT|AAG|AAA|TTT|ACT|TCG|AAA|GCG|TCT|TGA| (SEQ ID NO:201)
| ttt gac aaa ttc ttt aaa tga agc ttt cgc aga act - 5' (SEQ ID NO:202)

The paragraph on page 388, lines 24-29 (not counting blank lines), has been amended as follows:

+
| s | X | e | d | c | m | (SEQ ID NO:203)
| 70| 71| 72| 73| 74| 75|
| TCT|qfk|GAG|GAT|TGC|ATG|C (SEQ ID NO:204) 322
| agc *^m ctc cta acg tac gca ccc acc -5' (SEQ ID NO:205)
| Sph I | spacer |

The paragraph on page 389, last 5 lines, has been amended as follows:

| g | a | (SEQ ID NO:206)
| 80| 81|
| GGC|GCC| (SEQ ID NO:207)
| Bbe I |
| Nar I |

The paragraph on page 390, lines 23-28 (not counting blank lines) has been amended as follows:

+
| s | w | X | d | c | m | (SEQ ID NO:208)
| 70| 71| 72| 73| 74| 75|
| TCG|TGG|qfk|GAT|TGC|ATG|C (SEQ ID NO:209)
| agc acc *^m cta acg tac gcg acc tgc -5' (SEQ ID NO:210)
| Sph I | spacer |

The paragraph on page 391, last 5 lines, has been amended as follows:

| g | a | (SEQ ID NO:211)
| 80| 81|
| GGC|GCC| (SEQ ID NO:212)
| Bbe I |
| Nar I |

The paragraph on page 392, lines 21-25 (not counting blank lines), has been amended as follows:

| c | r | a | k | (SEQ ID NO:213)
| 61| 62| 63| 64|
|TGC|CGT|GCT|AAG|C (SEQ ID NO:214)
acg gca cga ttc gcg acc ggc (SEQ ID NO:215)
| Esp I | spacer |

The paragraph on page 393, last 5 lines, has been amended as follows:

| g | a | (SEQ ID NO:216)
| 80| 81|
|GGC|GCC| (SEQ ID NO:217)
| Bbe I |
| Nar I |

On page 397, after line 26, please add as a new paragraph:

(SEQ ID NO:218)

On page 398, after line 26, please add as a new paragraph (SEQ ID NO:219)

The paragraph on page 401, lines 16-19 has been amended as follows:

| S | . | . | . | (SEQ ID NO:220)
|131|132|133|134|
|TCT|TAA|TGA|TAG| GGTTACC- 486
| BstE II |

The paragraph on page 401, lines 26-27, has been amended as follows:

aTCGA (GACctgcaggcatgc)-3' (SEQ ID NO:221)
(SalI) from pGEM polylinker

The paragraph on page 404, lines 16-19, has been amended as follows:

| S | . | . | . | (SEQ ID NO:222)
 | 131|132|133|134|
 | TCT|TAA|TGA|TAG| GGTTACC-
BstE II

486

The paragraph on page 404, lines 26-27, has been amended as follows:

aTCGA GACctgca GGTCGACC ggcatgc-3' (SEQ ID NO:223)
 |SalI|

The paragraph on page 410, lines 27-30, has been amended as follows:

| S | . | . | . | (SEQ ID NO:224)
 | 131|132|133|134|
 | TCT|TAA|TGA|TAG| GGTTA/CC-
BstE II

The paragraph on page 410, lines 37-38, has been amended as follows:

a/(TCGA),-3' (SEQ ID NO:225)
(Sal I)

At the bottom of page 413, please add the following new paragraph:

For oligos #801-808 and 810-817, see SEQ ID NOS:226-241 in Table 105.

The paragraph on page 414, lines 3-11 (not counting blank lines) has been amended as follows:

Overlap Sequences	Junction	Tm
AGGCTTACTATGAAG	802:817	42. (SEQ ID NO:242)
TGTCCTTCGCTCG	803:816	42. (SEQ ID NO:243)
CTATTCTACAATGC	804:815	40. (SEQ ID NO:244)
AACAACTTTAAATCG	805:814	38. (SEQ ID NO:245)
CCTTCAATTCTCTGC	806:813	44. (SEQ ID NO:246)
CGTTGGTGCTACC	807:812	42. (SEQ ID NO:247)
CCAGTCTAA:GCC	808:811	42. (SEQ ID NO:248)

The paragraph on page 415, lines 1 to end, has been amended as follows:

Table 105: Individual sequences of Oligonucleotides 801-817.

Olig #801 (68 bases)

5'-ggcTTAcAc TTTATgcTTc cggcTcgTAT AATgTgTggA ATTgTgAgcg cTcAcAATTg AgcTcAgg-3' (SEQ ID NO:226)

Olig #802 (67 bases)

5'-AggCTTAcTA TgAAgAAATc TcTggTTcTT AAggctAgcg TTgcTgTcgC gAcccTggTA cctATgT-3' (SEQ ID NO:227)

Olig #803 (70 bases)

5'-TgTccTTcgc TcgTccggAT TTcTgTcTcg AgccAccATA cAcTgggccc TgcAAAGcgc gcATcATccg-3' (SEQ ID NO:228)

Olig #804 (67 bases)

5'-cTATTcTAc AATgcTAAAG cAggcccTgTg ccAgAccTTT gTATAcggTg gTTgcccTgc TAAAgcgT-3' (SEQ ID NO:229)

Olig #805 (76 bases)

5'-AAcAAcTTTA AATcgcccgA AgATTgcATg cgTAccTgcg gTggcgccgc TgAAggTgAT gATccggccA Aggcgg-3' (SEQ ID NO:230)

Olig #806 (67 bases)

5'-ccTcAATTc TcTgcAAgct TcTgcTAccg AgTATATTgg TTAcgcgTgg gccATggTgg TggTTAT-3' (SEQ ID NO:231)

Olig #807 (69 bases)

5'-cgTTggTgcT AccATcgggA TcAAACtgTT cAAgAAgTTT AcTTcgAAgg cgTcTTAATg ATAaggTTA-3' (SEQ ID NO:232)

Olig #808 (38 bases)

5'-ccAgTcTAAG cccgcCTAAT gAgcgggCTT TTTTTTA-3' (SEQ ID NO:233)

The paragraph on page 416, lines 1 to end, has been amended as follows:

Table 105: Individual sequences of Oligonucleotides 801-817.

(continued)

Olig #810 (29 bases)

5'-TcgATAAAAAA AAAAgccccgc TcATTAGgc-3' (SEQ ID NO:234)

Olig #811 (69 bases)

5'-gggCTTAgAc TggTAAcccT ATcATTAAgA cgcctTcgAA gTAAACTTcT TgAAcAgTTT gATcccgAT-3' (SEQ ID NO:235)

Olig #812 (65 bases)

5'-ggTAgcAccA AcgATAAccA ccAccATggc ccAcgcgTAA ccAATATAcT cggTAgcAgA AgcTT-3' (SEQ ID NO:236)

Olig #813 (76 bases)

5'-gcAgAgAATT gAAggccggcc TTggccggAT cATcAccTTc Agcggccca

ccgcAggTAc gcATgcAATc
TTcggc-3' (SEQ ID NO:237)

Olig #814 (67 bases)
5'-cgATTAAAG TTgTTAcgcT TAgcAcggcA AccAccgTAT AcAAAGgTcT
ggcAcAggcc TgcTTA-3' (SEQ ID NO:238)

Olig #815 (72 bases)
5'-gcATTgTAGA AATAgcggAT gATgcgcgcT TTgcAgggcc cAgTgTATgg
TggcTcgAgA cAgAAATccg Ga-3' (SEQ ID NO:239)

Olig #816 (65 bases)
5'-cgAgcgAAGg AcAAcATAgg TAccAgggTc gcgAcAgcAA cgcTAgccTT
AAgAAccAgA Gattt-3' (SEQ ID NO:240)

Olig #817 (63 bases)
5'-cTTcATAgTA AgccTccTgA gcTcATTgT gAgcgcTcAc AATTccAcAc
ATTATAcgAg ccggAAgc-3' (SEQ ID NO:241)

The paragraph on page 417, lines 1 to end, has been
amended as follows:

Table 106: Signal Peptides

PhoA	M K q s t i a l a l l p l l f t p v t K A / R T... (17)
	(SEQ ID NO:144)
MalE	M K I K T G A R i l a l s a l t t m m f s a s a l a / K I... (18)
	(SEQ ID NO:150)
OmpF	M M K R n i l a v i v p a l i v a g t a n a /a E ... (19)
	(SEQ ID NO:251)
Bla	M S I Q H F R v a l i p f f a a f c l p v f a /h p... (>18)
	(SEQ ID NO:252)
LamE	M M I T L R K l p l a v a v a a g v m s a q a m a /v D ... (19)
	(SEQ ID NO:253)
Lpp	M K A T K l v l g a v i l g s t l l a g /c s... (>17)
	(SEQ ID NO:154)
gpIII	M K K l i f a i p l v v p f y s h s /a E T V E ...
	(16) (SEQ ID NO:255)
gpIII-PPTI	M K K l i f a i p l v v p f y s g a / R P D ... (15)
	(SEQ ID NO:156)
gpVIII	M K K S L V L K a s v a v a t l v p m l s f a /a E G D D ...
	(16) (SEQ ID NO:257)
gpVIII-BPTI	M K K S L V L K a s v a v a t l v p m l s f a / R P D ... (15)
	(SEQ ID NO:158)
gpVIII'	M K K s l v l l a s v a v a t l v p m l s f a /a E G D D ...
	(21) (SEQ ID NO:258)

At the bottom of page 420, please add the following new paragraph:

(SEQ ID NO:260)

The paragraph on page 421, lines 10-14, has been amended as follows:

/ Cleavage site
↓
V V P F Y S H S A E T V (SEQ ID
NO:261)
11 12 13 14 15 16 17 18 19 20 21 22
1609 GTT GTT CCT TTC TAT TCT CAC TCC GCT GAA ACT GTT-3' (SEQ
ID NO:262)

The paragraph on page 421, lines 26-30, has been amended as follows:

/ cleavage site
↓
v v p f y s G A a e t v (SEQ ID
NO:263)
11 12 13 14 15 16 17 18 19 20 21 22
1609 gtt gtt cct ttc tat tct GGC Gcc gct gaa act gtt-3' (SEQ
ID NO:264)

The paragraph on page 423, line 18-20, has been amended as follows:

G A a e t v e s (SEQ ID NO:265)
77 78 79 80 81 82 83 84
GGC Gcc gct gaa act gtt GAA AGT

At the bottom of page 424, please add the following new paragraph:

(SEQ ID NO:266)

The paragraph on page 427, line 16-19, has been amended as follows:

	S		.		.		.		(SEQ ID NO:268)
	131		132		133		134		
	TCT		TAA		TGA		TAG		<u>GGTTACC-</u> <u>BstE II</u>

At the bottom of page 427, please add the following new paragraph:

(SEQ ID NO:267)

The paragraph on page 430, lines 24-27, has been amended as follows:

	S		.		.		.		(SEQ ID NO:270)
	131		132		133		134		
	TCT		TAA		TGA		TAG		<u>GGTTACC-</u> <u>BstE II</u>

The paragraph on page 430, lines 34-35, has been amended as follows:

aTCGA GACctgca GGTGAC-3' (SEQ ID NO:269)
|SalII|

The paragraph on page 433, lines 3-5, has been amended as follows:

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
fM	K	K	L	L	F	A	I	P	L	V	V	P	F	Y
ATG	AAA	AAA	TTA	TTA	TTC	GCA	ATT	CCT	TTA	GTT	GTT	CCT	TTC	TAT
<----- gene III signal peptide -----														

The paragraph on page 437, lines 13-15, has been amended as follows:

481 482 483 484 485
N K E S . (SEQ ID NO:272)
AAT AAG GAG TCT TAA (SEQ ID NO:271)